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ANTIBACTERIAL BEHAVIOR OF LEUKOCYTES IN NORMAL SUBJECTS FOR CHEDIAK-HIGASHI DISEASE AND PELGER-HUET'S ANOMALY

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ANTIBACTERIAL BEHAVIOR OF LEUKOCYTES IN NORMAL SUBJECTS FOR CHEDIAK-HIGASHI DISEASE AND PELGER-HUET'S ANOMALY

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ABSTRACT. A comparison is made of the behavior of lymphocytes in being transformed into "blasts" under the influence of phytohemagglutinin and phagocytes as regards its effects on multiplication of S. aureus and S. marcescens in 6 healthy individuals, one with Pelger-Huet's anomaly and another with Chediak-Higashi's disease. /15*

The blast transformation and the nucleolar index, whose constancy is known in normal blood samples, were normal in the case of Pelger-Huet's anomaly and abnormal in Chediak-Higashi's disease, in which there was less lymphocytic response. This suggested a behavior similar to that found in chronic lymphoid leukemia.

While Pelger-Huet's anomaly, like normal blood, showed a tendency toward staphylococcal destruction from the first through the third hours and Serratia continued to multiply, in Chediak-Higashi's disease, the former barely appeared before the third hour. However, Serratia proliferated abundantly initially, falling off in the end, a phenomenon which we feel may explain the fact that Chediak-Higashi's disease sufferers may be invaded by pyogenic germs and potentially pathological saprophytes.

Traditionally leukocytic functions have been studied microscopically by /5 studying the phagocytosis discovered by Metchnikoff in 1892, its metabolic

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*Numbers in the margin indicate pagination in the original foreign text.

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activity in relation to the former (Karnovsky, 1962) and the richness and activity of enzymes (Fiessinger, 1923).

The processes cited above have been further elaborated upon with the discovery of the role of leukocytic lysosomes (De Duve, 1955), the dynamic exploration of leukocytes in culture media as simplified by Osgood and Kripaehne (1955) and through the use of phytogemagglutinin, a substance which stimulates mitosis derived from the bean phaseolus vulgaris (Nowell, 1960), as well as the study of the effects of phagocytosis on the survival and multiplication of bacteria proposed by Miller and Buckler (1968).

The subject of the present work is to report on the exploration of the two functions mentioned on the background of several normal cases: Pelger-Huet's anomaly (Microphotograph No. 1) and in a patient with Chediak-Higashi's disease (Microphotograph No. 2).

Material and Methods /6

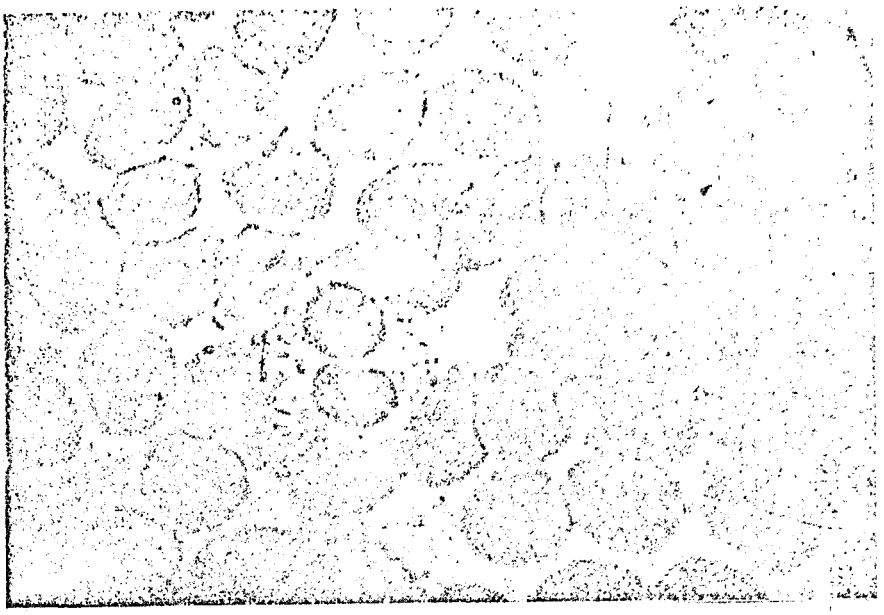
1. Lymphocyte culture and determination of blast formation.

The short-term culture is made according to Moorhead (1960): Venous blood is taken and rendered incoagulable by heparine; through sedimentation at 37°C, the plasma layer is separated with the leukocytes which are dispersed in a TC 199 medium with and without phytohemagglutinin.

After 72 hours' incubation at 37°C, the cells are recovered by centrifuging at 800 r.p.m., washed, and stained for classification.

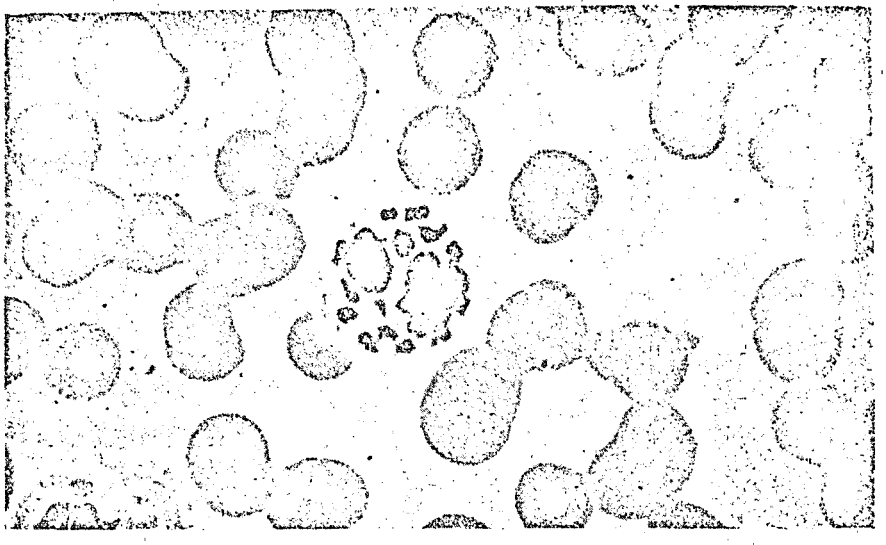
We use the Gonzalez Guzman stain (1935), which consists of suspending the leukocytes in an equal number of drops of methyl alcohol and distilled water with Giemsa coloring (10-12 drops per ml of alcohol-water mixture). The results of this coloring can be seen in Microphotographs 3 and 4. The corpuscles scarcely appear to be hemolyzed, the mononuclear cytoplasm appears lightly stained blue, the nucleus is a slightly darker blue or

MICROPHOTOGRAPH NO. 1



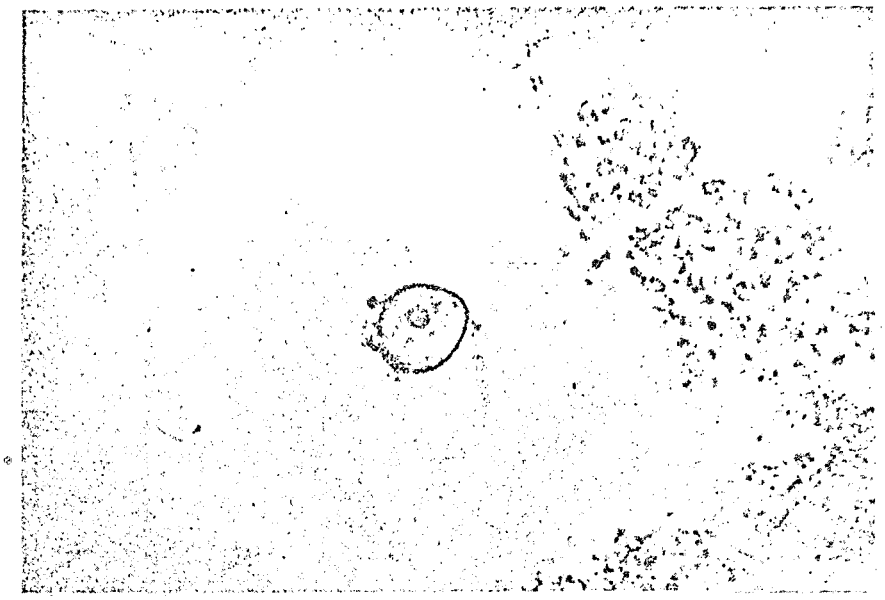
Pelger-Huet Anomaly

MICROPHOTOGRAPH NO. 2



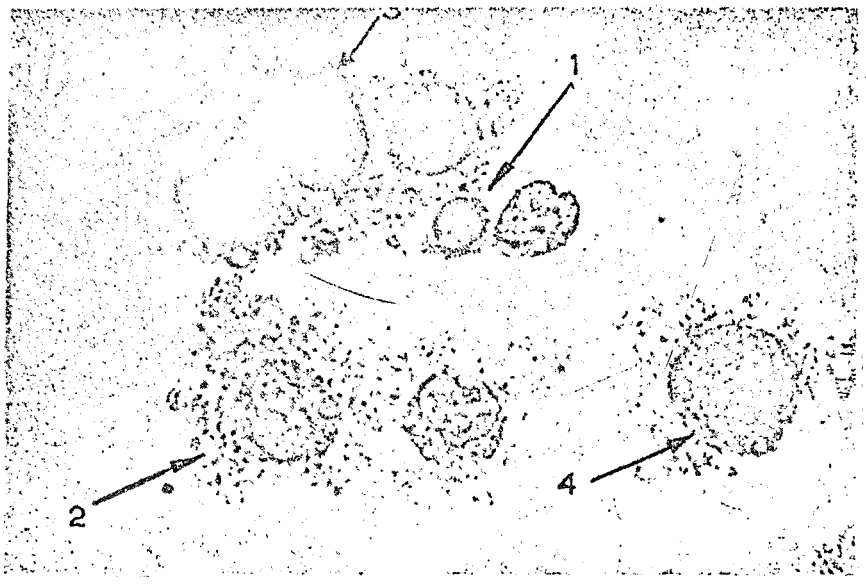
Chediak-Higashi's Disease

MICROPHOTOGRAPH NO. 3



Small form with one nucleolus

MICROPHOTOGRAPH NO. 4



1 - Small form with two nucleoli; 2 - Large form (blast) with one nucleolus; 3 - Large form (blast) with two nucleoli; 4 - Large form (blast) with three nucleoli.

bluish purple, and the nucleolus is dark purple; the granulocytes retain their well-known affinity for Romanowsky derived stains.

The mononuclears (mostly lymphocytes) may be classified as small (comparable size to that of corpuscles) and large (larger than corpuscles) with the latter category containing most of the cytoplasm, the occasionally slightly shortened nucleus, and the greatest number of nucleoli. Taking into account the last element, we counted the nucleoli in the 100 mononuclear elements, and derived the nucleolar index according to Gonzales and Guzman, or the number of nucleoli in 100 cells counted.

We strongly recommend the method described when tritium marking is required because of the constant normal values obtained and shown in the table constructed after examining the blood of 50 normal subjects (Table 1).

We should like to call attention to the quite clear differences which result from stimulation by phytohemagglutinin and the very small variation in the averages as supported by the low values for standard deviation (S.D.).

2. Study of intracellular survival and development after phagocytosis: /8

To make this study, we followed the Miller Buckler (1968) method with slight modifications which consists of incubating the leukocytes with suspensions of Serratia marcescens (pigmentless variation) and Staphylococcus aureus (positive coagulase) at 37°C for one hour; centrifuging at slow speeds, separating the supernatant, killing the extracellular germs with penicillin and streptomycine, and taking the aliquot part of the sediment with phagocytes and germs, the same as those destroyed by treatment with a hypotonic solution and dispersed in agar. The colonies are counted initially (0 hour) and after 1 and 3 hours of incubation.

TABLE 1. pH STIMULATION. AVERAGES OF 50 NORMAL SAMPLES*

	small lymphocytes %	S.D.	large lymphocytes % blasts	S.D.	nucleolar index	S.D.
no PH	99.64	0.70	0.36	0.20	99	1.29
with PH	48.26	2.50	51.74	2.60	108	1.73

* 100 cells counted in each case.

Results:

The Pelger-Huet anomaly showed a 48% blastoid transformation and a nucleolar index of 109, which were within the range of normal values, while in Chediak-Higashi's disease, phytohemagglutinin produced only 26% blasts with a nucleolar index of 108. The first figure was low, and the second — normal.

As far as the study of the influence of phagocytes on germ multiplication is concerned, we make a count for comparison with the behavior of six samples of normal subjects, in five of which the behavior was normal with inhibition for S. aureus after the first hour and multiplication of S. marcescens from the same moment on (Figures 1 and 2).

In Pelger-Huet's anomaly the behavior of the germ-phagocyte complex was normal (Figure 3), but this was not the case in Chediak-Higashi's disease where S. aureaus continued to proliferate for the first hour and the proliferation of S. marcescens increased considerably, with later decreases for both subsequently (Figure 4).

Discussion

We feel that the studies carried out yielded information of clinical and pathological interest. In the first place, they are consistent with

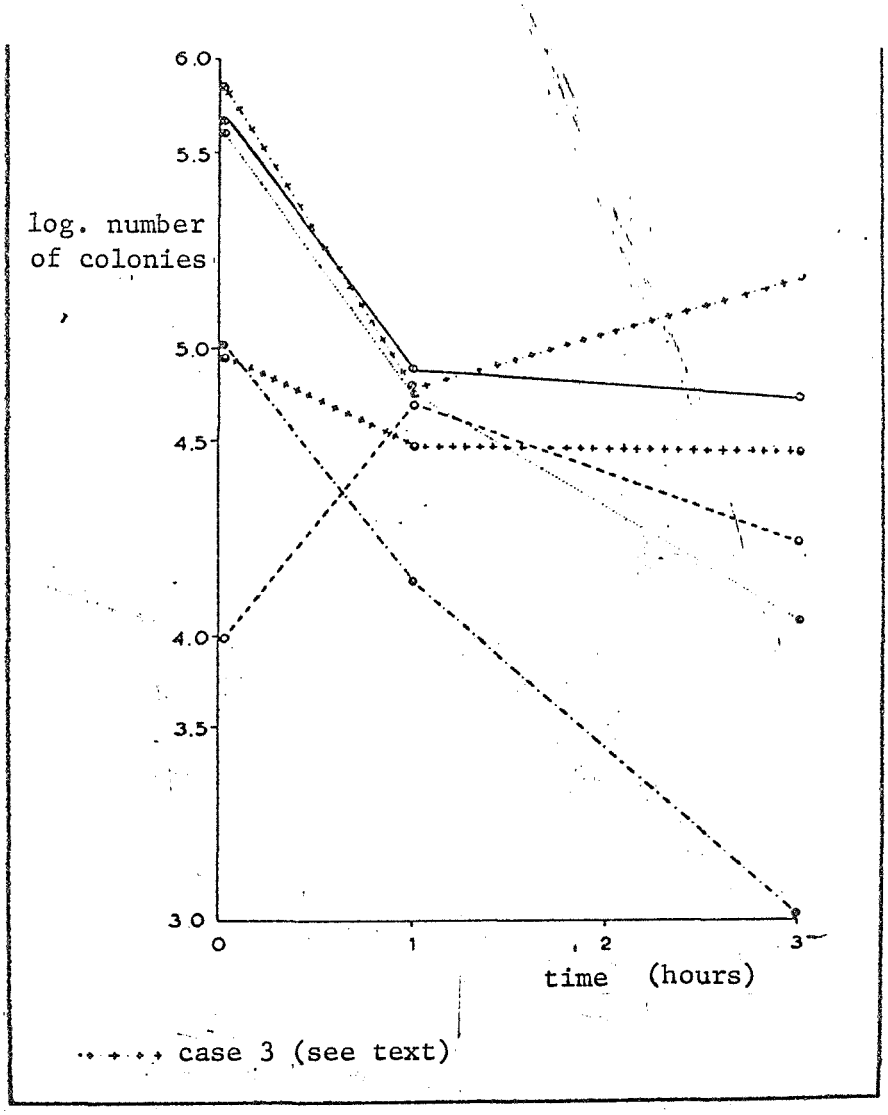


Figure 1. Survival and development of staphylococcus aureus in leukocytes of 6 normal subjects.

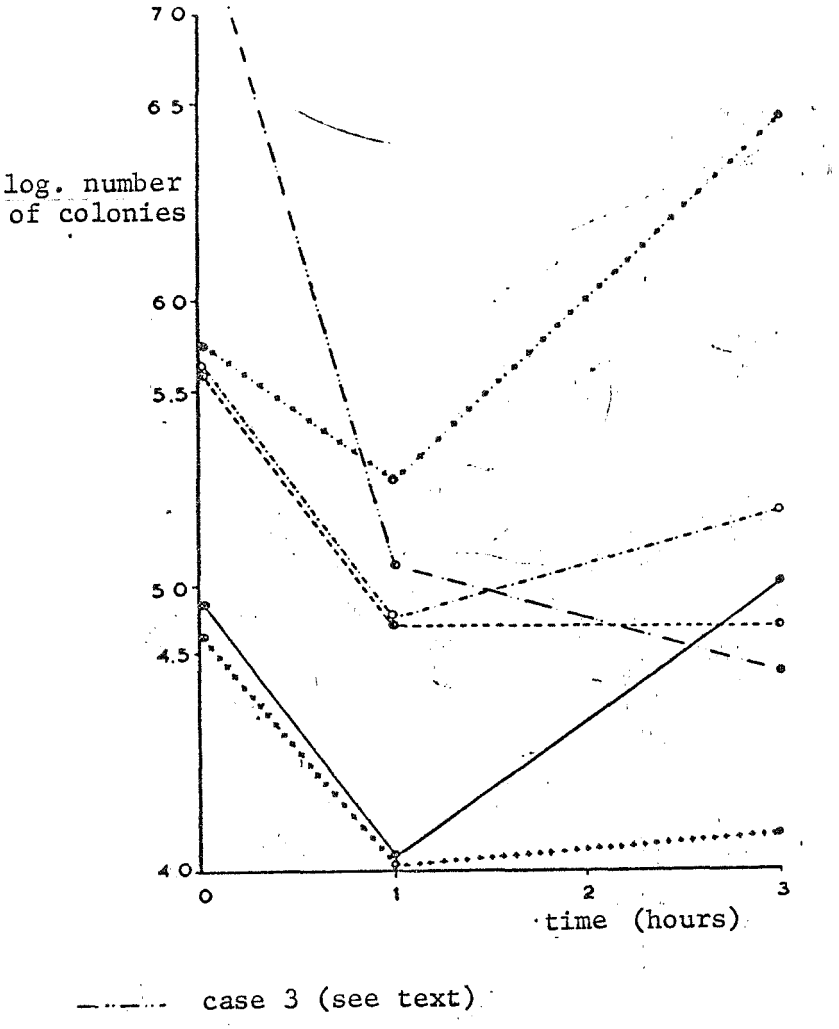


Figure 2. Survival and development of Serratia marcescens in leukocytes of 6 normal subjects.

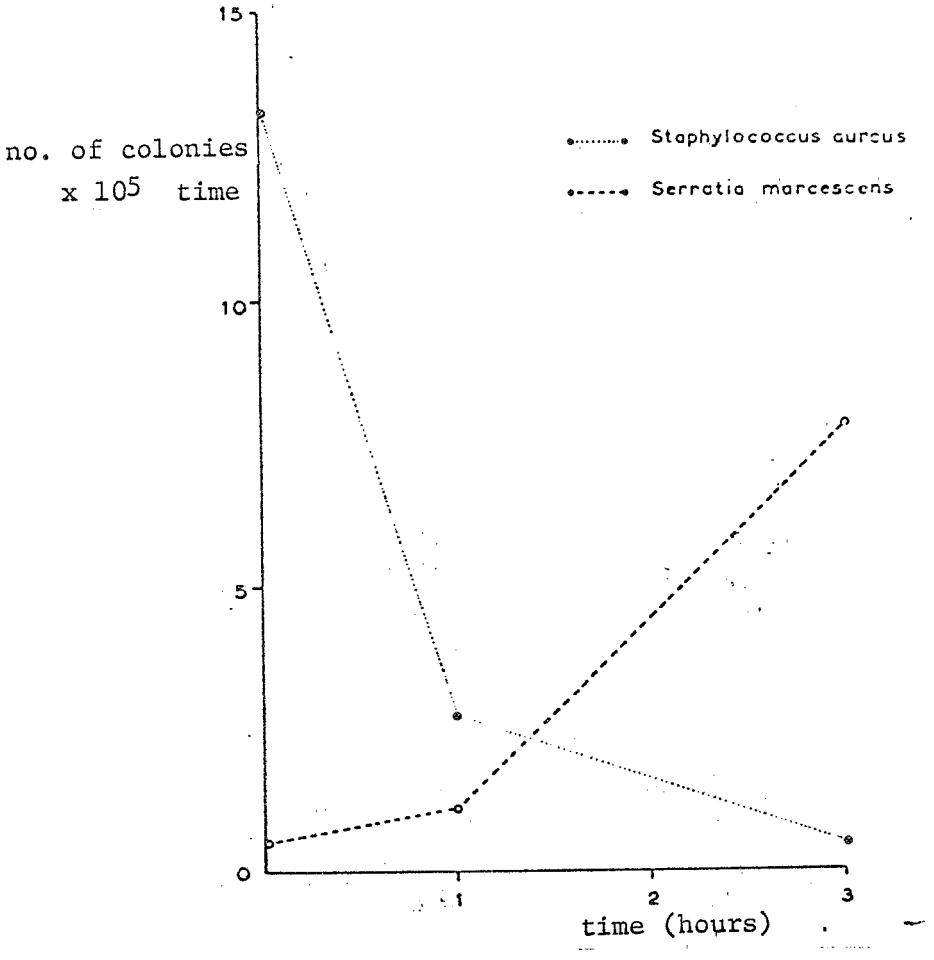


Figure 3. Pelger-Huet's anomaly; survival and development in leukocytes.

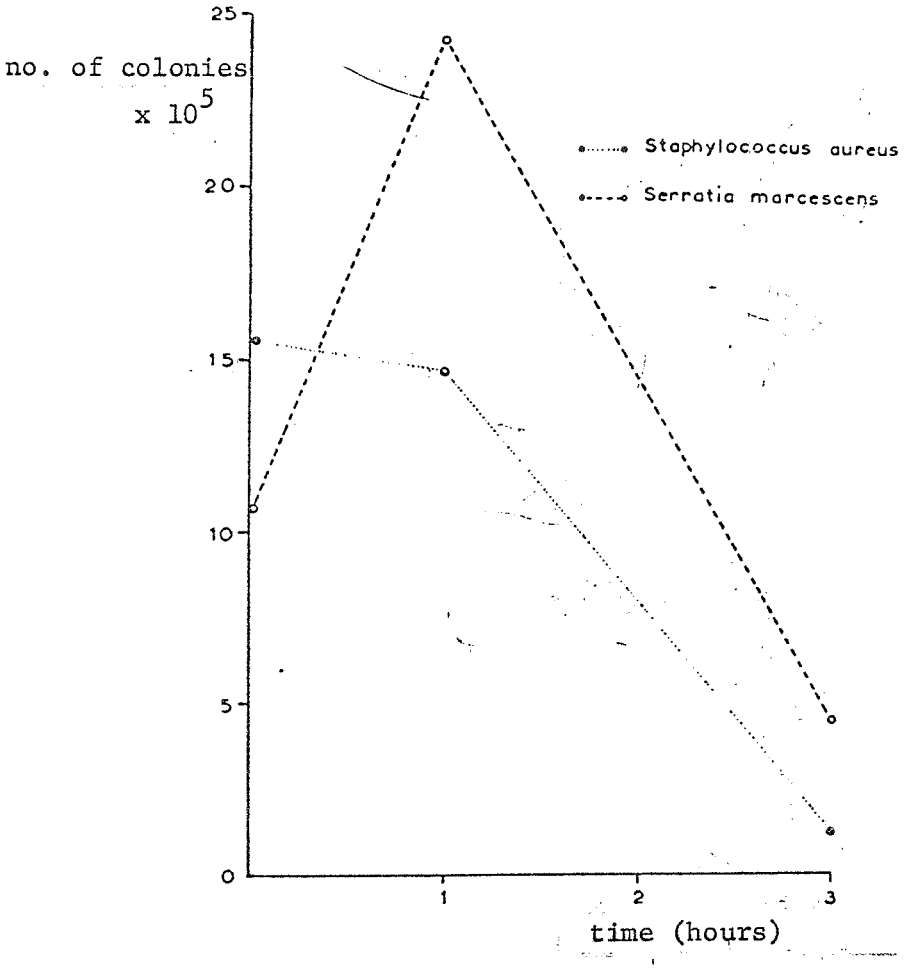


Figure 4. Chediak-Higashi's disease; survival and development in leukocytes.

the clinical benign nature of Pelger-Huet's anomaly, so often born out as regards the normal behavior of heterozygotes (lack of predisposition for infections, lack of tendency toward leukemia or lymphoma, normal survival). They also support the demonstrated malignancy of Chediak-Higashi's disease summed up in the propensity of these patients to evolve in the direction of lymphoma (Efrati and Jonas, 1958) and in their predisposition for repeated serious infections. These can be explained, according to our research, by the incapability of their phagocytes to rapidly annihilate such aggressive microbes as S. aureus or even to exhibit an immediate bacteriocidal power with regard to germs which, like S. marcescens, are not aggressive, but certainly pathological.

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